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Characterization of Antibodies Mediating Protection and Cure of *Trypanosoma musculi* Infection in Mice

DANIEL S. WECHSLER,[1,2]* AND PATRICIA A. L. KONGSHAVN[1-3]

Montreal General Hospital Research Institute[1] and Department of Physiology[2] and McGill Cancer Centre, [3] McGill University, Montreal, Quebec, Canada H3G 1Y6*

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Plasma samples were collected from mice infected with *Trypanosoma musculi* at different times postinfection and administered to naive recipient mice either before or during *T. musculi* infection. The protective and curative activities of these plasma samples were shown to increase as the time of collection postinfection increased; plasma collected at 14 days postinfection was partially protective and partially curative, whereas that collected at 28 days postinfection was completely protective and curative. The curative activity was labile to heat treatment (30 min at 56°C), whereas the protective activity was heat stable. Additional kinetic parameters relating to the efficacy of protection were investigated. Evidence is presented that both activities are immunoglobulin in nature. Protein A-Sepharose chromatography indicated that the activities are associated with the immunoglobulin G2a or immunoglobulin G3 subclasses of immunoglobulin G. The curative antibody appears to be intrinsically heat labile, since heat treatment of a purified immunoglobulin preparation abolished the ability to cure. Studies on the mechanism of parasite elimination from blood suggest that the process not only requires antibody but is also complement dependent.

Trypanosoma musculi, a natural parasite of *Mus musculus*, produces a characteristic, self-limiting infection in mice which lasts for approximately 3 weeks (28, 29, 31). After inoculation with trypanosomes, there is a short lag phase, followed by an exponential rise in the number of parasites in the blood. The reproductive rate then declines and the parasitemia stabilizes at a plateau level, with almost the entire population of visible trypanosomes becoming adult in form. Between the second and third weeks of infection, the plateau phase is terminated and the parasites are eliminated from the blood over a 3- to 4-day period.

In both T-cell- (4, 23, 31) and B-cell-deprived (30) mice, the parasitemia stabilizes after the initial growth phase, yet elimination of trypanosomes does not occur. These observations suggest that the development of T-cell-dependent humoral immunity is important for host defense and essential for parasite elimination and cure of the mouse. Serum from immune hosts passively transferred to naive recipients has been shown to have a transitory protective effect on the latter (30, 31) or to be fully protective (with no ensuing parasitemia) (2), and mice born to normal mothers and nursed by immune ones were also initially completely protected upon trypanosome challenge (3). However, passive transfer of immune serum from a recovered donor to an infected mouse has generally been ineffective in mediating cure, i.e., elimination of a preexisting parasitemia. Although Taliaferro in 1938 (27) reported a curative effect of immune serum when administered to mice infected 3 to 7 days previously, the scant data make it difficult to interpret his exact findings. Similarly, the data on which Büngener (5) based his cure of infected mice are somewhat ambiguous. More recently, investigators have been unable to cure an infected mouse with the passive transfer of serum from mice naturally recovered from *T. musculi* infection (2, 29, 31). Thus, although fairly good evidence could be obtained for an activity in immune serum that fully or partially protected a naive recipient from becoming infected, a role for humoral immunity in eliminating the parasites in *T. musculi* infection has been in considerable doubt (2). We have recently demonstrated (33) that plasma collected from mice 1 week after clearance of parasitemia cures a trypanosomal infection in recipient mice as well as protects a naive recipient mouse from being infected. Further, the first of these activities is labile to heat treatment, whereas the second is heat stable. We now present a characterization of the kinetic parameters of these curative and protective activities and demonstrate unequivocally that they are antibody in nature. In addition, evidence is presented suggesting that the antibody-mediated curative activity is dependent on the presence of complement component C3.

MATERIALS AND METHODS

Parasites. The Partinico II strain of *T. musculi*, originally provided by P. Viens, Département de Microbiologie et Immunologie, Université de Montréal, Québec, Canada, was used throughout. Parasites were passaged through mice at biweekly intervals.

Mice. Male and female C57BL/6 mice (Charles River Breeding Laboratories, Inc., St. Constant, Canada) 6 to 8 weeks old were used for experiments. Retired female C57BL/6 breeders 6 to 9 months old were used in the preparation of plasma.

Infection of mice. An inoculum of 10^4 viable *T. musculi* obtained by serial passage was administered intraperitoneally (i.p.) to mice.

Measurement of infection. Individual heparinized blood samples were taken from the retro-orbital sinus every 2 to 3 days postinfection (p.i.) or at longer intervals as indicated. If the level of parasitemia was low, infection was assessed by counting parasites on wet blood films. Blood (5 μl) was dispersed on a glass slide with a cover slip (22 by 40 mm). Parasites were enumerated microscopically with a 40× objective and a 10× ocular. When more than five parasites per high-power field were present, hemacytometer counts were

* Corresponding author.

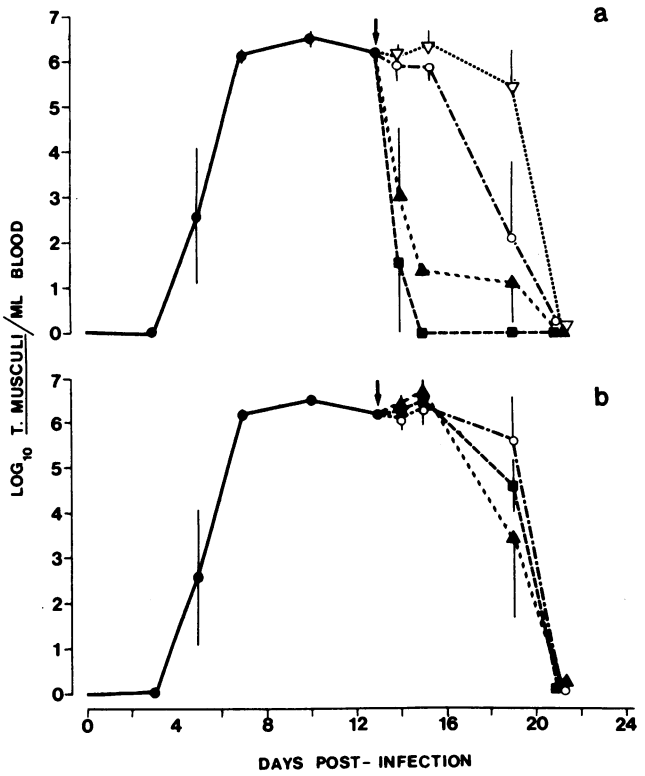

FIG. 1. Thermolabile curative activity in donor plasma from infected mice that was given to recipient mice during the course of *T. musculi* infection. C57BL/6 mice were inoculated i.p. with 10^4 trypanosomes and given 0.4 ml of plasma i.v. on day 13 (arrow). Plasma was taken from mice infected 14 (○), 19 (▲), or 28 (■) days previously or from noninfected mice (▽). (a) Freshly thawed plasma. (b) Plasma was HT at 56°C for 30 min. Each point represents the mean value of four or five mice ±1 standard error of the mean.

made of infected blood diluted 1:100 with saline containing 0.02% Formalin. The number of parasites per milliliter of blood was calculated and expressed as $\log_{10}$ values.

Plasma preparation. Plasma was obtained from mice infected 7, 14, 19, and 28 days before collection. Mice were bled by cardiac puncture with heparinized syringes. The plasma was separated from cellular components and stored at −20°C. Plasma collected from mice approximately 1 week after clearance of infection (28 days p.i.) is termed immune plasma (IP). Control, noninfected donors were used to prepare normal mouse plasma (NMP). Where required, plasma was heat treated (HT) by incubation in a 56°C water bath for 30 min to 2 h.

Plasma injection. Unless otherwise indicated, all injections of plasma were intravenous (i.v.) and were administered as a volume of 0.4 ml per mouse. Plasma was administered either before trypanosome inoculation or during the course of an established infection.

CVF. Cobra venom factor (CVF; Cedarlane Laboratories, Hornby, Canada), a decomplementing agent (6), was administered to mice in two injections. Mice received one dose of 10 U of CVF i.p. 24 h before injection of IP and another 10 U i.p. immediately before IP administration. Plasma was tested for the presence of C3 by the Ouchterlony double diffusion technique with rabbit anti-mouse C3 (Cappel Laboratories, Cochranville, Pa.) in the center well and dilutions of plasma in the outer wells. Agar immunodiffusion plates (Hyland Diagnostics, Malvern, Pa.) were placed in an incubator at 37°C for 2 h, followed by overnight incubation at 4°C.

Molecular sieve chromatography. Plasma was applied to an LKB Ultrogel ACA34 (IBF, Villeneuve-La-Garenne, France) column (1.6 by 90 cm) equilibrated in phosphate-buffered saline (pH 7.4). Fractions of 2.5 ml were collected, and the protein content of the fractions was calculated from measurement of absorbance at 280 nm, assuming an extinction coefficient of 1.4 optical density U/mg of protein per ml. Appropriate fractions were pooled, concentrated to original volumes in a protein concentrator (Amicon Corp., Lexington, Mass.), and dialyzed versus phosphate-buffered saline before injection into mice to assay activity.

Protein A-Sepharose. The procedures described by Ey et al. (8) and Seppälä et al. (25) were followed with minor modifications. Staphylococcal protein A covalently linked to Sepharose 4B was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden), swollen in phosphate-buffered saline (pH 8.0) with 0.02% sodium azide, and packed into a 10-ml glass pipette. The column (bed volume, 5 ml) was stored and used in a cold room at 4 to 5°C. A two-ml amount of 0.1 M phosphate buffer (pH 8.0) was added to 5 ml of plasma, and the pH was adjusted to 8.1. This material was applied to the column, and the column was rinsed with the pH 8.0 buffer until the optical density at 280 nm was less than 0.01. Material bound to the column was successively eluted with 0.1 M citrate-phosphate buffers of pHs 6.0, 4.5, and 3.5; each time the column was rinsed until no further material eluted. Fractions (2.5 ml) were collected, absorbances at 280 nm were measured, and fractions eluted with a given buffer were pooled and neutralized to pH 7.0 with 1 M Tris hydrochloride (pH 8.5). These pooled fractions were designated by the pH of elution, concentrated to the original plasma volume in a protein concentrator, and dialyzed versus phosphate-buffered saline.

Each fraction was tested for the presence of immunoglobulin G (IgG) subclasses or IgM by the Ouchterlony double immunodiffusion technique as described above with rabbit anti-mouse immunoglobulin reagents (Litton Bionetics, Willowdale, Canada, and Miles, Rexdale, Canada) in the center wells and serial doubling dilutions of fraction in the outer wells. The endpoint was read as the weakest titer of fraction which gave a detectable precipitin line.

Injection of fractions. Mice were injected with the above column fractions either before *T. musculi* inoculation or during an established infection. Injections were i.v. in a volume of 0.8 to 1.0 ml (2 to 2.5 times the volume of IP normally active) to compensate for losses during fractionation. Parasitemia levels were monitored after injections.

Statistical analysis. Statistical analysis was performed by using the Student *t* test (two tailed). *P* values of <0.05 were considered significant.

RESULTS

Thermolabile curative activity in donor plasma from infected mice. Plasma preparations from donors infected 14, 19, and 28 days previously were given to groups of recipient mice on day 13 of infection. The plasma from 14- and 19-day infected donors conferred some ability to eliminate the parasites, whereas the 28-day IP cleared the blood of trypanosomes in 24 to 48 h (Fig. 1a). IP has been demonstrated to clear parasitemia in as little as 3 h (unpublished observations). This is termed the curative activity of plasma. When the plasma preparations were HT for as little as 30 min

TABLE 1. Effect of supplementing HT plasma with complement

Treatment[a] (no. of mice)	Log_{10} trypanosomes per milliliter of blood ±1 SEM at day after plasma administration:		
	0	1	2
IP (7)	5.61 ± 0.07	0	0
HTIP (5)	5.80 ± 0.13	5.33 ± 0.32	5.47 ± 0.41
HTIP + NMP (11)	5.66 ± 0.07	5.33 ± 0.16	5.75 ± 0.13
IP + HTNMP (4)	5.61 ± 0.05	0	0

[a] Mice were treated with a 0.4-ml i.v. injection of IP or NMP. HTIP or HTNMP (0.4 ml) was mixed with 0.4 ml of non-HT plasma (NMP or IP, respectively) and administered in a 0.8-ml i.v. dose. All injections were performed on day 12 p.i.

at 56°C and tested as before, the curative activity was lost (Fig. 1b).

Reconstitution of HTIP with complement. To ascertain whether the heat lability of IP was related to complement inactivation, HTIP was reconstituted with fresh NMP as a source of complement and tested for curative activity. Parasitemia was cleared within 24 h in control groups receiving non-HT IP, whereas no such clearance occurred in mice receiving HTIP, even when supplemented with NMP (Table 1). Thus, abolition of curative activity in IP by heat treatment is apparently due to inactivation of a plasma component other than complement.

Kinetics of development of protective activity in plasma during the course of *T. musculi* infection. Groups of mice were inoculated with trypanosomes after administration of NMP or plasma taken from mice infected 7, 14, 19, or 28 days previously. A control group of infected mice received no treatment (NT). Parasitemias were followed in all mice, and the results are shown in Table 2. Plasma taken from 7-day-infected mice had no effect, but when taken at later times p.i., it lowered or completely abolished the parasitemia. The longer the duration of infection of the plasma donors, the greater was the degree of protection obtained, and IP (28 days) was fully protective, i.e., no parasites were detected at any day in the recipients. This antitrypanosomal activity found in the plasma of *T. musculi*-infected mice is termed protective.

When the above plasma preparations were HT for 2 h at 56°C and tested in the same manner, essentially the same results were obtained as before, with the parasitemias becoming patent on the same days p.i. When analyzed for statistical significance, it was found that the numbers of trypanosomes in the blood of mice that had received either freshly thawed or HT plasma were not significantly different ($P > 0.05$). This was true for any day that the parasitemia was measured.

Protective effect of plasma on infection with different doses of *T. musculi*. Table 3 demonstrates that the protective activity of IP was considerably diminished when the infecting dose of trypanosomes was increased by two orders of magnitude. As before, the parasitemia observed in mice pretreated with HTIP was not significantly different from that seen in mice pretreated with IP ($P > 0.05$) for any day that the parasitemia was measured.

Kinetics of disappearance of protective activity of transferred IP. Groups of mice were pretreated with IP at different times before trypanosome infection. IP was only fully protective when given on the day of infection (Fig. 2). However, partial protection was clearly evident when IP was administered 3 days before infection, and some protective activity remained even when IP was administered 2 weeks before *T. musculi* inoculation, as evidenced by the decreased plateau level of parasitemia.

Fractionation of IP. An initial partial purification of IP with an Ultrogel ACA34 molecular sieve chromatography column revealed the curative activity to be in a fraction of plasma containing material with a molecular weight of less than 200,000 (data not shown). Further purification of activity was obtained by applying a sample of plasma to a column of protein A-Sepharose and collecting fractions containing material eluted by citrate-phosphate buffers of decreasing pH. The elution profiles of Fig. 3 compare the protein A-Sepharose separations of IP and NMP, demonstrating a significantly larger amount of protein in the pH 4.5 peak. The relative concentrations of immunoglobulin isotypes in each fraction were assessed by Ouchterlony immunodiffusion analysis. The pH 4.5 fraction contained mostly IgG2a and IgG3, and no other fractions had significant amounts of these immunoglobulin subclasses present (Table 4). The pH 4.5 eluate brought about a dramatic reduction in parasitemia (curative effect) within 24 to 48 h of administration, whereas the pH 8.0, 6.0, and 3.5 fractions did not markedly affect the course of infection (Fig. 4). Heat-treatment of the fractions before administration abolished any curative effect.

Figure 5 shows the effect of administering fractions to naive mice before *T. musculi* inoculation to assay the protective activity of the fractions. It is evident that the pH

TABLE 2. Effect of heat treatment on protective activity of IP taken from mice at various times p.i. and used to pretreat mice before infection with *T. musculi*

Donor plasma used to pretreat mice[a]	Log_{10} trypanosomes per milliliter of blood (mean of 4 or 5 mice ±1 SEM) at day p.i.[b]:						
	5	7	10	12	14	18	20
None	3.23 ± 1.52	4.90 ± 0.02	5.55 ± 0.09	5.55 ± 0.09	5.64 ± 0.06	4.35 ± 0.35	4.70 ± 0.10
7 day	4.00 ± 1.40	4.83 ± 0.11	5.60 ± 0.12	5.67 ± 0.03	5.64 ± 0.06	5.40 ± 0.00	4.70 ± 0.26
7 day, HT	3.08 ± 1.03	3.72 ± 1.24	5.79 ± 0.13	5.70 ± 0.17	5.76 ± 0.24	5.63 ± 0.55	5.26 ± 0.36
14 day	0	3.00 ± 1.00	4.65 ± 0.06	4.79 ± 0.17	5.07 ± 0.08	4.35 ± 0.22	4.13 ± 0.16
14 day, HT	0	3.85 ± 0.09	4.77 ± 0.17	5.17 ± 0.16	4.85 ± 0.16	4.75 ± 0.19	4.70 ± 0.40
19 day	0	0	4.00 ± 0.17	4.16 ± 0.08	4.43 ± 0.22	4.63 ± 0.15	4.30 ± 0.38
19 day, HT	0	0	1.93 ± 1.11	3.16 ± 1.05	3.35 ± 1.13	4.50 ± 0.07	4.50 ± 0.40
28 day	0	0	0	0	0	0	0
28 day, HT	0	0	0	0	0	0	0
NMP	2.77 ± 1.34	4.82 ± 0.06	5.53 ± 0.09	5.65 ± 0.05	5.58 ± 0.06	5.23 ± 0.15	5.18 ± 0.23
NMP, HT	3.90 ± 0.10	4.71 ± 0.18	5.33 ± 0.13	5.40 ± 0.14	5.28 ± 0.12	4.54 ± 0.54	4.15 ± 0.45

[a] Donor plasma was collected from mice 7, 14, 19, and 28 days p.i. as indicated. Half of the pooled plasma samples were HT at 56°C for 2 h. A 0.4-ml dose of plasma was given to each recipient mouse i.v. 1 h before infection with 10^4 trypanosomes i.p. One group received NMP.

[b] All trypanosomes were cleared by day 22.

TABLE 3. Effect of administering plasma to mice immediately before infection with different doses of *T. musculi*

Pretreatment of mice[a]	Inoculating parasite dose	Log_{10} trypanosomes per milliliter of blood (mean of 5 mice ±1 SEM) at day p.i.:					
		2	4	6	8	14	18
NT	10^4	0	0.93 ± 0.93	4.55 ± 0.41	6.07 ± 0.15	6.14 ± 0.25	5.37 ± 0.03
IP	10^4	0	0	0	0	0	0
HTIP	10^4	0	0	0	0	0	0
NT	10^6	0.70 ± 0.70	4.98 ± 0.08	5.50 ± 0.14	5.48 ± 0.17	6.54 ± 0.36	0
IP	10^6	0	2.57 ± 1.29	3.95 ± 1.39	5.23 ± 0.62	5.97 ± 0.52	2.23 ± 2.23
HTIP	10^6	0	4.79 ± 0.06	4.98 ± 0.19	5.37 ± 0.38	4.30 ± 0.27	1.85 ± 1.85

[a] Mice were either NT or injected i.v. with 0.4 ml of IP or HTIP 1 h before i.p. infection with 10^4 or 10^6 parasites.
[b] All trypanosomes were cleared by day 22.

4.5 fraction significantly delayed the onset of parasitemia, as well as decreased the plateau level, whereas the other fractions had marginal, if any, effect on the course of parasitemia.

Decomplementation of mice before IP administration. CVF is known to bring about in vivo depletion of complement component C3 as well as late-acting components (6). Groups of mice received CVF before IP administration on day 12 of *T. musculi* infection, and parasitemia was subsequently monitored. Control mice receiving IP alone cleared the infections within 24 h, whereas mice receiving IP preceded by CVF failed to eliminate the trypanosomes (Table 5). Analysis of plasma by Ouchterlony double immunodiffusion analysis revealed that the plasma from CVF-treated mice was severely depleted of C3, showing only a faint line at a dilution of 1:2. Plasma from non-CVF-treated mice, on the other hand, showed a line at a dilution of 1:64 (data not shown).

DISCUSSION

The present findings confirm and extend our earlier demonstration of both curative and protective activities in the blood of mice infected with *T. musculi* (33). The results presented in Fig. 1 and Table 2 demonstrate the gradual appearance of both activities during the course of infection. Whereas the presence of protective activity in serum has been consistently demonstrated (2, 30, 31), the curative activity of transferred serum has been much less readily apparent (2, 5, 27, 29, 31). Albright and Albright (2) collected sera on days 8, 12, 16, and 20 of *T. musculi* infection and administered them to infected recipient mice. They were, however, unable to effect any form of cure by passive transfer of any of these sera. The discrepancy between their results and the present findings may be explained by considering that the efficacy of IP in curing infection depends on the relative amounts of curative factor therein and the level of parasitemia. MacAskill et al., in studies on *T. brucei* (18) and *T. congolense* (19, 34), suggested that acute, fatal infections of trypanosomiasis are the result of the inability of the host to achieve effective levels of circulating antibody against a rapidly replicating trypanosome clone. Indeed, they too were unable to cure *T. brucei*-infected mice by passive transfer of hyperimmune serum, attributing the failure to inadequate levels of antibody (18). Their results indicate that the trypanotolerance of C57BL/6 mice (to *T. congolense*) depends on the superior humoral response of this mouse strain (19). Further, we have shown (D. S. Wechsler and P. A. L. Kongshavn, manuscript in prepara-

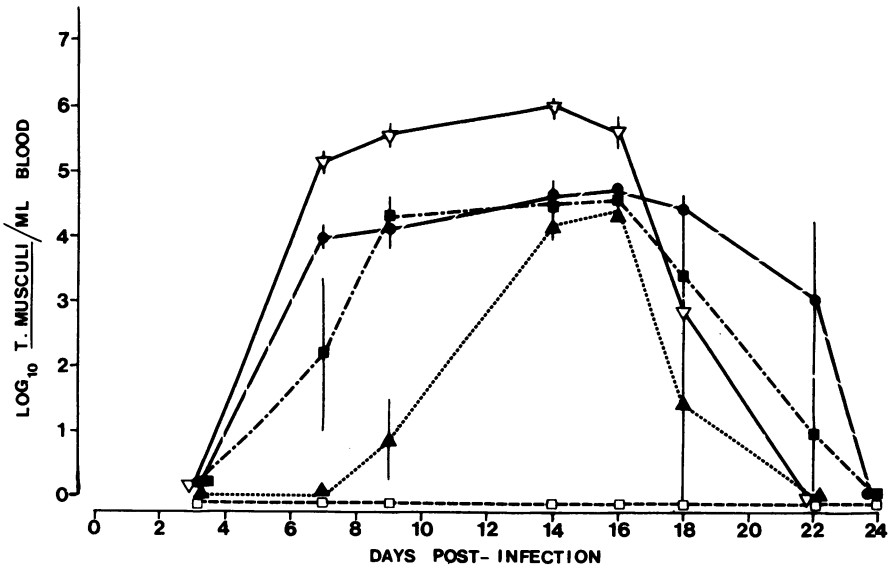

FIG. 2. Kinetics of disappearance of transfused protective activity in plasma during the course of *T. musculi* infection. C57BL/6 mice were pretreated i.v. with 0.4 ml of IP (taken 28 days p.i.) 14 (●), 7 (■), or 3 days (▲) before infection or on the day of infection (□). A dose of 10^4 trypanosomes was given i.p. Control infected mice received no pretreatment (▽). Each point represents the mean value of four or five mice ±1 standard error of the mean.

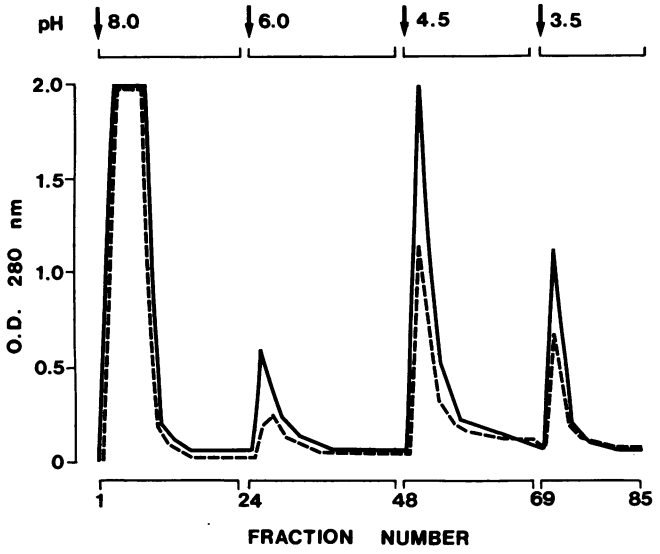

FIG. 3. Protein A elution profile of IP (———) and NMP (- - -). Elution was stepwise with buffers of decreasing pH. Fractions were collected and pooled as indicated and concentrated to original plasma volumes. At 280 nm, 1.4 absorbance units represent 1 mg of protein per milliliter. O. D., optical density.

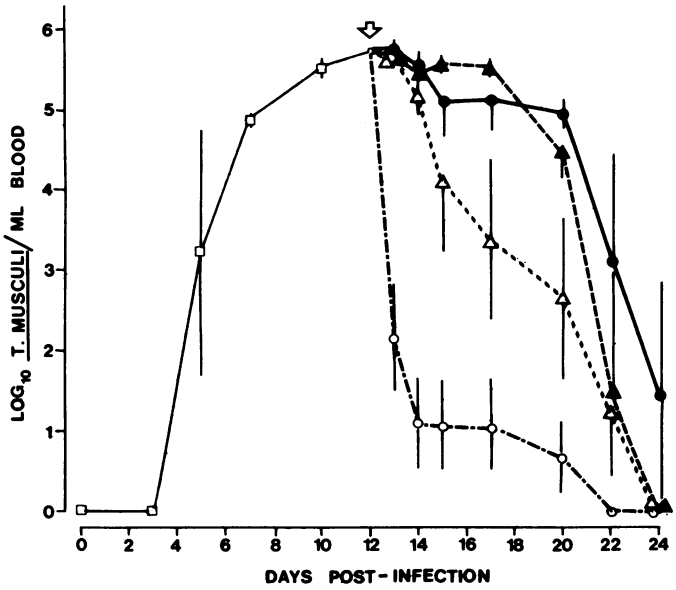

FIG. 4. Curative activity of protein A-eluted IP fractions. On day 12 p.i. (arrow), C57BL/6 mice were given 0.8-ml i.v. injections of IP fractions eluted from protein A with buffers of decreasing pH (8.0 [▲], 6.0 [△], 4.5 [○], 3.5 [●]). Heat treatment of fractions before injection abolished any curing effect. Each point represents the mean value of five or six mice ±1 standard error of the mean.

tion) that IP is able to effect a cure in mouse strains in which parasitemia is relatively low. Albright and Albright (2), on the other hand, performed their serum transfer experiments in A and C3H mice known to have higher plateau levels of parasitemia than C57BL/6 mice (1). Moreover, their injections were performed on days 7 and 10 with smaller amounts of serum. Presumably, the transferred plasma or serum is more efficacious when given later in the infection, at a time when endogenously produced curative activity is also present in the recipient (Fig. 1). In this case, transferred and endogenously produced curative activity would have an additive effect.

In addition, the results extend our previous finding that the curative activity is labile to heat treatment, whereas the protective activity is stable (33). Although the two activities may be distinguished on the basis of their sensitivity to heat treatment, this does not necessarily imply that the factors responsible are different entities. Rather, the observed differential heat sensitivity of the activities may be a quantitative phenomenon. It is likely that heat inactivation is a gradual process, in which more and more factor is rendered ineffective with increasing exposure to heat. If so, it might be expected that after 0.5 h of treatment, there could still be some residual amount of active factor present, sufficient to eliminate 10^4 trypanosomes and protect the host, but insufficient to cure the latter of the plateau load of 10^6 parasites. Indeed, when mice were infected with 10^6 *T. musculi* organisms, the protective effect previously seen even with non-HTIP was severely diminished (Table 3). Again, this may be explained by a lack of sufficient factor to cope with the higher number of parasites.

TABLE 4. Analysis of IP fractions by Ouchterlony double immunodiffusion

pH of fraction	Endpoint of isotype[a]				
	IgM	IgG1	IgG2a	IgG2b	IgG3
8.0	1:16				
6.0		1:128			
4.5		1:8	1:128	1:1	1:32
2.5		1:1	1:2	1:128	1:1

[a] Endpoint read as weakest titer of fraction giving a precipitin line.

The fact that there is still some protective activity 2 weeks after IP injection (Fig. 2) suggests a fairly long biological half-life of the protective activity.

Given an initial evaluation of the kinetic parameters of the biological activities of curing and protection, we began to investigate the nature of the factors. Previous studies concerning the humoral reponse to *T. musculi* have been equivocal, with a number of reports indicating no role for antibody in the elimination of parasites (2, 29). This is surprising in light of experiments from our laboratory (30), which show clearly that cure of *T. musculi* infection is a B-cell-dependent process. Thus, an initial partial purification of IP was carried out with molecular sieve chromatography. The curative activity was found to be associated with a fraction of molecular weight less than 200,000, which is consistent with the size of IgG. In subsequent studies, advantage was taken of the technique proposed by Ey et al. (8) and modified by Seppälä et al. (25) for the isolation of IgG subclasses with protein A-Sepharose. These workers and others (17) have shown that mouse IgG is adsorbed by protein A-Sepharose at pH 8.0, whereas other proteins do not bind to any significant extent. The different subclasses of IgG may be sequentially eluted with buffers of decreasing pH, viz., IgG1 elutes at pH 6.0, IgG2a and IgG3 elute at pH 4.5, and IgG2b elutes at pH 3.5. IP was thus fractionated accordingly, and Fig. 4 shows that the curative activity does not appear in the flow-through fraction but is associated with the pH 4.5 fraction. This provides persuasive evidence that the curative factor is antibody in nature. Moreover, it was confirmed that the pH 4.5 fraction contained mostly subclasses IgG2a and IgG3 and that no other fraction had significant amounts of either subclass (Table 4). Based on these observations and on the aforementioned elution characteristics, the curative

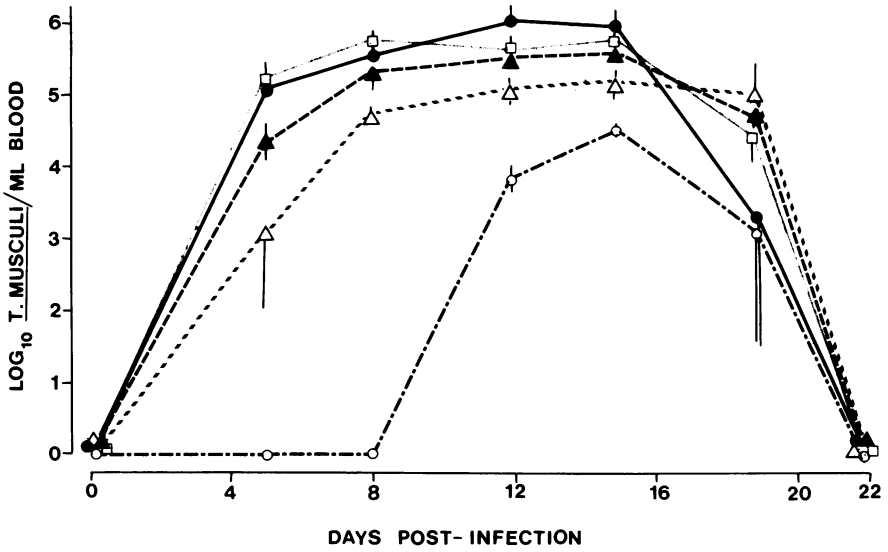

FIG. 5. Protective effect of protein A-eluted IP fractions on the course of infection in naive recipients. C57BL/6 mice were inoculated i.p. with 10^4 trypanosomes 1 h after i.v. treatment with 0.8-ml plasma equivalents of IP fractions eluted from protein A with buffers of decreasing pH (8.0 [▲], 6.0 [△], 4.5 [○]). NMP (●) was administered and there was an NT (□) control Each point represents the mean value of four or five mice ±1 standard error of the mean.

antibody seems to be associated with either IgG2a or IgG3. The slight decrease in parasitemia observed with the pH 6.0 fraction (Fig. 4) may be due to a partial effectiveness of IgG1 in causing parasite elimination, or it may be the result of contamination of the fraction with IgG2a or IgG3. Indeed, although IgG2a was not detected in this fraction by the Ouchterlony technique, small amounts were detected in the pH 6.0 fraction by the Mancini radial immunodiffusion technique (unpublished observations). Both Ey et al. (8) and Seppälä et al. (25) pointed out a similar slight contamination problem.

The protective activity was also associated with the pH 4.5 fraction of IP (Fig. 5), indicating that it too may be mediated by IgG2a or IgG3. As discussed above, the same antibody may be responsible for both the curative and protective activities.

The apparent restriction of functional anti-trypanosome curing activity to one or two subclasses is of interest in light of findings by Perlmutter et al. (22) that murine anti-group A streptococcal carbohydrate antibodies are largely restricted to the IgG3 subclass as well as the work of Majarian et al. (21), demonstrating the curing of *Plasmodium yoelii* infections with an IgG3 monoclonal antibody.

As mentioned previously, the in vivo curing activity of IP is sensitive to heat treatment. Initially, the nature of the lability was unclear; either the activity was intrinsically heat sensitive or some accessory plasma component was labile, most likely complement. The addition of fresh NMP as a source of complement did not reverse the effects of heat treatment, indicating that heating affected some other component (Table 1). The heat lability of the isolated pH 4.5 activity confirms that the curative antibody therein is intrinsically heat sensitive; polyacrylamide gel electrophoresis of this fraction demonstrates no contamination by other components, i.e., only immunoglobulin is present (unpublished observations). Although IgE is generally assumed to be the sole heat-labile murine immunoglobulin (15), it has not been shown to bind to protein A-Sepharose; it is thus unlikely to be present in the pH 4.5 fraction and is unlikely to be responsible for the observed specific cure. Earlier work has mentioned the aggregation of immunoglobulins on exposure to heat or alkali (13, 26), yet no loss of biological activity was reported. Indeed, serum samples are routinely heat inactivated to eliminate the effects of complement without subsequent loss of immunoglobulin activity. A clue to the understanding of the mechanism of heat inactivation is provided by the work of Rousseaux-Prévost et al. (24) on rat IgE inactivation. They showed that heating induces non-disulfide-linked polymerization of IgE molecules and proposed that steric hindrance does not allow interaction between C-terminal domains of the H chain and the IgE receptor. Henney and Stanworth (13) proposed a similar aggregation of IgG via formation of intermolecular disulfide bonds in the Fc region. Assuming that an antitrypanosomal antibody molecule bound to a parasite via the antigen-binding site must then interact with a cell via the Fc region to effect clearance (see below), it is evident that blocking the Fc region will lead to a loss of biological activity. It should be pointed out that the loss of in vivo activity will not necessarily be paralleled by a loss of in vitro activity (agglutination, enzyme-linked immunosorbent assay) if the antigen-combining region is left unaltered.

Finally, the results presented in Table 5 demonstrate that although antibody is necessary to bring about trypanosome elimination, it alone is not sufficient. Our data indicate that C3 must be present for IP to bring about clearance of parasitemia. Jarvinen and Dalmasso (16) have shown that rendering mice C3 deficient during the plateau phase prolongs *T. musculi* infections by interfering with parasite

TABLE 5. Effect of CVF treatment on curing activity of IP

Treatment[a] (no. of mice)	Log_{10} trypanosomes per milliliter of blood (±1 SEM) at day after IP administration:		
	0	1	2
NT (7)	5.68 ± 0.08	0	0
CVF (5)	5.78 ± 0.08	5.87 ± 0.18	6.06 ± 0.26

[a] Mice were infected with *T. musculi* and either NT or treated with 20 U of CVF. IP was administered on day 12 p.i.

elimination. Similar findings were obtained with *T. brucei* by MacAskill et al. (20), who showed that CVF treatment prevented immune clearance in passively immunized mice. Although CVF also depletes the later complement components, it is unlikely that these are involved in parasite elimination; Dusanic (7) and others (16) have shown that the course of *T. musculi* infection in inbred, C5-deficient mice is not significantly different from that in normocomplementemic mice and have concluded that complement-dependent, antibody-mediated cytolysis is probably not a major mechanism for the elimination of *T. musculi* by the infected mouse. Again, MacAskill et al. (20) demonstrated similar findings for *T. brucei* infections in mice.

Taken together, these findings suggest a murine model for the elimination of *T. musculi* based on the cooperative interactions of both antitrypanosomal antibody and C3. Other data from our laboratory and elsewhere (2, 32) suggest that a cell (macrophage? platelet?) is involved as well. For instance, it is well known that antigens complexed with both antibody of the IgG class and C3b form complexes that bind avidly to macrophages by means of multiple Fc and C3b receptor interactions (12). Similar in vitro and in vivo requirements have been ascertained for a number of trypanosome species (9, 10, 11, 14, 18).

In conclusion, the curative activity appears to be an intrinsically heat-labile member of the IgG class of immunoglobulins, which increases in concentration or avidity or both during the course of the infection, and which appears also to require complement (C3 but not C5) to be effective in mediating parasite elimination. Studies are currently under way to determine the predominant IgG subclass mediating the phenomenon as well as to investigate the cellular mechanism(s) of clearance.

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